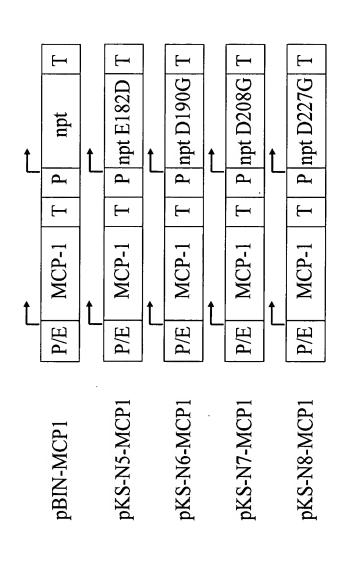
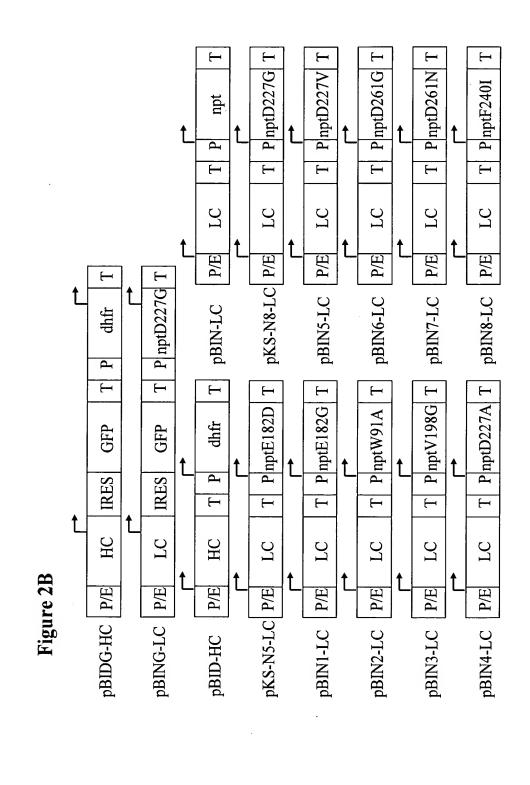
$\vdash$ npt dhfr Ь Ь Ţ  $\vdash$  $\vdash$  $\vdash$ dhfr GFP GFP npt IRES IRES Ь Ь Τ P/E (mcs) (mcs) (mcs) P/E (mcs) P/E( P/E pBING pBIDG pBID pBIN

Figure 1

Figure 2A





## Figure 3

.91 . Ala Gly Arg Asp Trp Leu Leu Gly Glu Ala Glu Leu Phe Ala Arg Leu Lys Ala Ser Met

5'-gagctattccagaagtagtgaggaggtttt.....GCG GGA AGG GAC TGG CTG TTA TTG GGC GAA.....GCC GAA CTG TTC GCC AGG CTC AAG GCG AGC ATG
3'-ctcgataaggtcttcatcatcactcctccgaaaa.....CGG CCT CCG CTG ACC GAC GAT AAC CCG CTT.....CGG CTT GAC AAG CGG TCC GAG TTC CGC TCG TAC 198 GC W91Afor

Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp Cys Gly Arg Leu D190Gfor T E182Dfor

S F S GAC c D190Grev A E182Drev

Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala

A F240Ifor C/T/G D227Afor/D227Vfor/D227Gfor

261 Phe Tyr Arg Leu Leu Asp Glu Phe Phe Stop

... AAG ATA G<u>GG GAA CTG CTC AAG AAG ACT cgc....gttcgctgcgggttggacggtag</u>t....gaaa<u>actaaatattccctaaaacggc</u>taa-5'

" D261Nrev IC49 ...ITC TAT CGC CTT CTT GAC GAG TTC TTC TGA gcg....caagcgacgcccaacctgccatca....cttttgatttataagggattttgccgatt-3′ G D261Gfor c D261Grev

 $G_{r}^{C}$ AAC TGG => CCG; Glu182Gly: GAG => GGT; Glu182Asp: GAG => GAT; Asp190Gly: GAT => Val198Gly: GTG => GGG; Asp208Gly: GAC => GGC; Asp227Val: GAT => GTT; Asp227Ala: GAT => Â Phe240Ile: TTC => ATC; Asp261Gly: GAC => GGC; Asp261Asn: GAC Asp227Gly: GAT => GGT; rrp91Ala:

Figure 4

AQGRPVLFVK	91 WLLLGEVPG
CSDAAVFRLS	ATTGVPCAAY LDVVTEAGRD
GYDWAQQTIG	ATTGVPCAAV
SPAAWVERLF	QDEAARLSWL A'
MIEQDGLHAG SPAAWVERLF GYDWAQQTIG CSDAAVFRLS	TDLSGALNE
$\leftarrow$	51

IERARTRMEA	198	ACL PNIMNEN	Motif 1	LVLYGIAAPD	
CPFDHQAKHR	182 190	GEDLVVTHGE	240	ELGGEWADRE	
RRLHTLDPAT		FARLKASMPD	227	IALATRĎIAE	
EKUSIMADAM		EHQGLAPAEL		GRESGFIDGG RIGVADRYQD IALATRDIAE ELGGEWADRE LVLYGIAAPD	261
101 DLLSSHLAPA EK <u>V</u> SIMADAM RR <u>L</u> HT <u>L</u> DPAT <b>C</b> PFDHQAKHR IERARTRMEA		151 GLVDQDDLDE EHQGLAPAEL FARLKASMPD GEDLVVTHGD ACLPNIMVEN	208	GRESGFIDCG	Motif 2
101		151		201	

SQRIAFYRLL DEFF Motif 3

251

NPT-Asp227Gly, 400 µg G418 NPT-Glu182Asp, 400 µg G418 NPT-WT, 800 µg G418 NPT-WT, 400 µg G418 Figure 5 0.000 0.300 0.400 0.100 0.200 Titre and specific productivity

☐ spezif. Prod. (pg/Zelle\*Tag) ■ Titer (mg/L)

Figure 6A

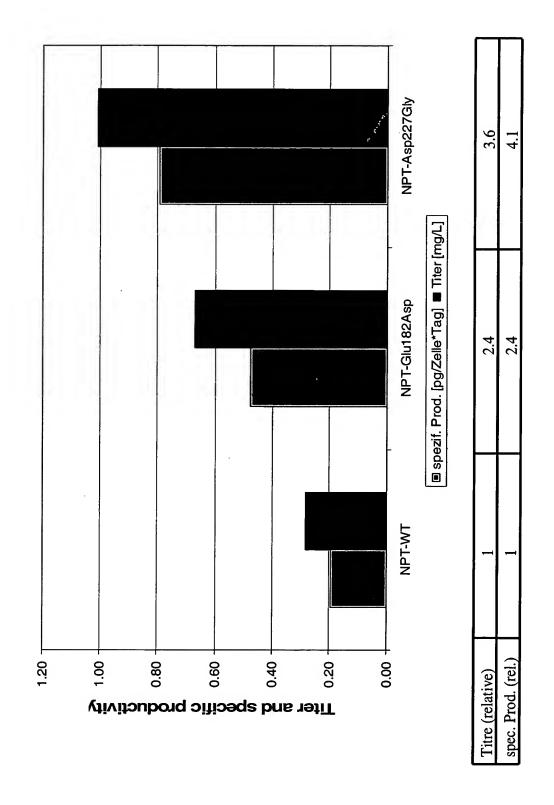


Figure 6B

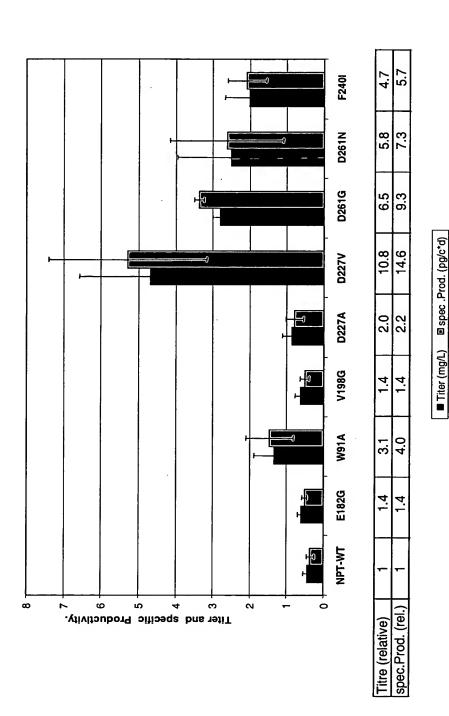
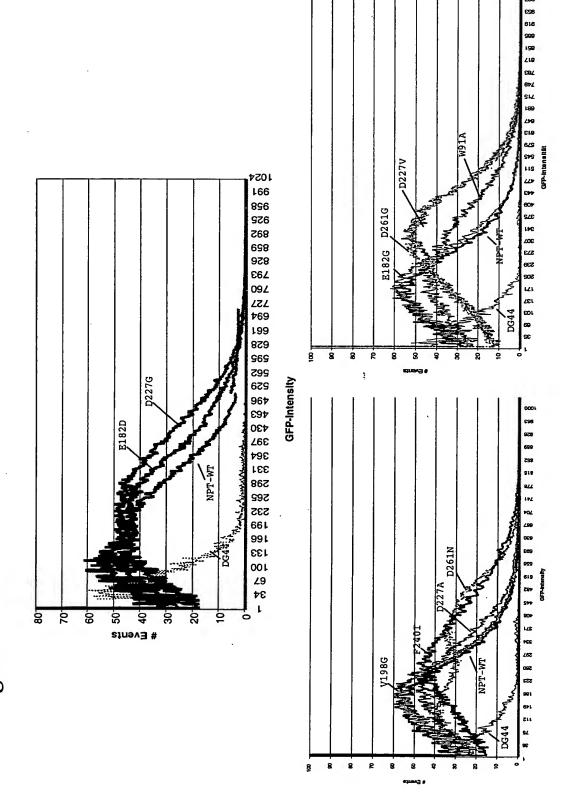


Figure 7



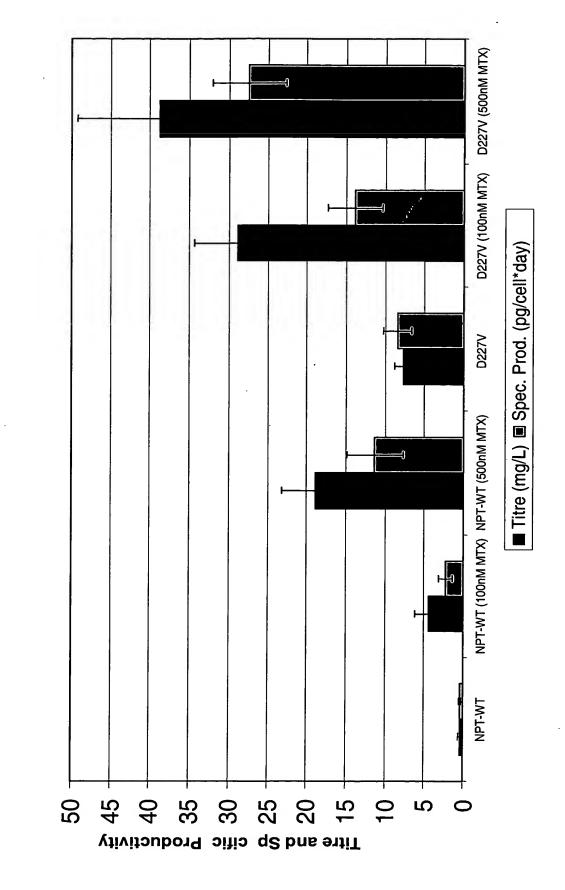


Figure 8

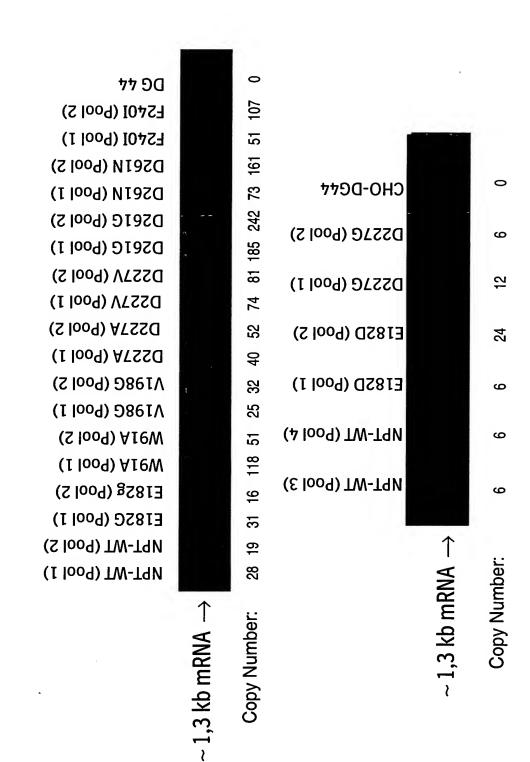
**Phosphocellulose** 

Nitrocellulose

Figure 9B

NPT-Variant	% Enzyme activity (WT-NPT = 100%)
NPT-Wildtype	100.0
Trp91Ala	53.2
Glu182Gly	25.8
Glu182Asp	22.0
Val198Gly	61.9
Asp227Ala	26.9
Asp227Val	16.4
Asp227Gly	30.0
Phe240Ile	23.4
Asp261Gly	3.1
Asp261Asn	1.5

Figure 10



Pool 5 Pool 5 Pool 5 Pool 5 Pool 5 Pool 5 Pool 8 Po Figure 11 0.00 12.00 -10.00 8.00 6.00 4.00 2.00 Titre and specific productivity

■ Titer [mg/L] 

Spec. Prod. [pg/Cell / Tag]

Pool 8 Sort 1 100 nM MTX 1.4 Port School & Garage Million Charles Pool 8 Sort 1 Pool 8 Pool 5 Sort 1 100 nM MTX Pool 5 Sort 1 Pool 5 25.00 20.00 15.00 10.00 0.00 5.00 Titre and specific Productivity

■ Titer [mg/L] 

Spec. Prod. [pg/Cell/ Tag]

Figure 12

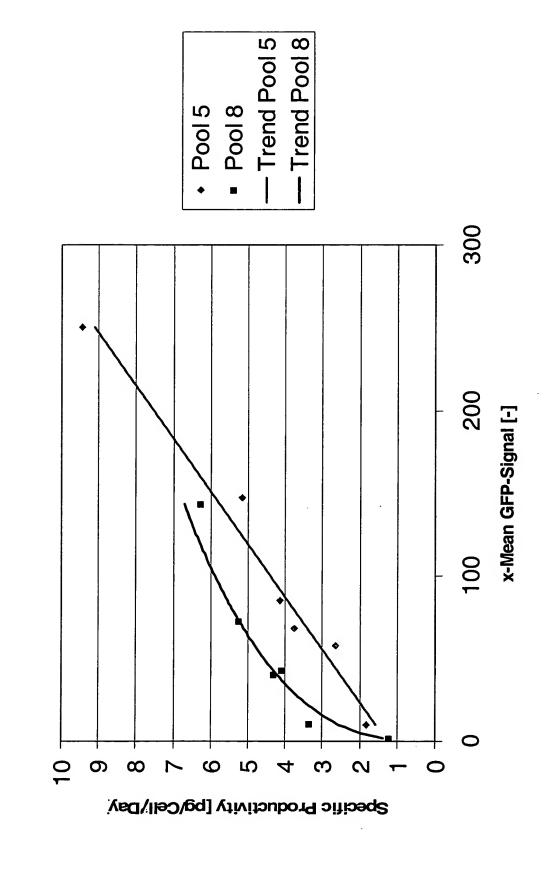


Figure 13